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Article

The Forebrain Song System Mediates Predictive Call Timing in Female and Male Zebra Finches

Highlights

- Zebra finches can learn to modify the timing of their innate calls
- Birds dynamically adjust call timing in anticipation of complex rhythm patterns
- Non-singing females exhibit strong call timing plasticity
- The song control system is necessary for predictive call timing and precision

Authors

Jonathan I. Benichov, Sam E. Benezra, Daniela Vallentin, Eitan Globerson, Michael A. Long, Ofer Tchernichovski

Correspondence

jonathan.benichov@gmail.com

In Brief

Benichov et al. show that zebra finches predictively adjust the timing of unlearned calls in response to rhythmic call patterns. Female zebra finches, who don't sing, show better call timing plasticity than males. In both sexes, the forebrain song system is required for this learning, suggesting a role for this circuit in interactive vocal coordination.

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Current Biology
Article

The Forebrain Song System Mediates Predictive Call Timing in Female and Male Zebra Finches

Jonathan I. Benichov,[1,2,*] Sam E. Benezra,[3,4] Daniela Vallentin,[3,4] Eitan Globerson,[5,6] Michael A. Long,[3,4] and Ofer Tchernichovski[1]
[1]Department of Psychology, Hunter College, City University of New York, New York, NY 10065, USA
[2]Doctoral Program in Biology, The Graduate Center, City University of New York, New York, NY 10016, USA
[3]Neuroscience Institute and Department of Otolaryngology, New York University Langone Medical Center, New York, NY 10016, USA
[4]Center for Neural Science, New York University, New York, NY 10003, USA
[5]Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 52900, Israel
[6]Jerusalem Academy of Music and Dance, Jerusalem 91904, Israel
*Correspondence: jonathan.benichov@gmail.com
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SUMMARY

The dichotomy between vocal learners and non-learners is a fundamental distinction in the study of animal communication. Male zebra finches (*Taeniopygia guttata*) are vocal learners that acquire a song resembling their tutors', whereas females can only produce innate calls. The acoustic structure of short calls, produced by both males and females, is not learned. However, these calls can be precisely coordinated across individuals. To examine how birds learn to synchronize their calls, we developed a vocal robot that exchanges calls with a partner bird. Because birds answer the robot with stereotyped latencies, we could program it to disrupt each bird's responses by producing calls that are likely to coincide with the bird's. Within minutes, the birds learned to avoid this disruptive masking (jamming) by adjusting the timing of their responses. Notably, females exhibited greater adaptive timing plasticity than males. Further, when challenged with complex rhythms containing jamming elements, birds dynamically adjusted the timing of their calls in anticipation of jamming. Blocking the song system cortical output dramatically reduced the precision of birds' response timing and abolished their ability to avoid jamming. Surprisingly, we observed this effect in both males and females, indicating that the female song system is functional rather than vestigial. We suggest that descending forebrain projections, including the song-production pathway, function as a general-purpose sensorimotor communication system. In the case of calls, it enables plasticity in vocal timing to facilitate social interactions, whereas in the case of songs, plasticity extends to developmental changes in vocal structure.

INTRODUCTION

The acoustic structures of most animal vocalizations are not learned. The ability to match vocal sounds to sensory templates is rare, although recent studies show evidence for vocal learning in a growing number of species [1–3]. Vocal coordination is much more common, having been recognized in many species that are vocal non-learners, such as cicadas [4], frogs [5], and marmosets [6]. Here we investigate the hypothesis that vocal coordination is mechanistically, and perhaps evolutionarily [7, 8], linked to vocal learning.

We examine call coordination in zebra finches, taking advantage of their sexual dimorphism. Males are vocal learners that acquire their songs by imitation, but females are vocal non-learners that cannot sing and produce only innate calls [9, 10]. Short affiliative contact calls (“tets” and “stacks”) are the most frequently produced vocalizations among both sexes [9, 11]. Although the forebrain song system is not required for producing short calls [12] or responding to them [10], birds appear to show plasticity in the timing of their calls. As in many social species that are vocal non-learners, the temporal patterns of calls produced by a group of zebra finches can be highly structured [13, 14], which may reflect social bonds [15–17], reproductive state [18], and social hierarchy (J. Benichov and O. Tchernichovski, 2012, Soc. Neurosci., abstract) [19].

To test how male and female zebra finches learn to synchronize their calls, we presented them with a vocal robot that we initially programed to produce calls in a simple isochronous pattern. This allowed the bird to exchange back-and-forth (antiphonal) calls with the robot. We then programmed the robot to produce call patterns designed to disruptively interfere with this communication channel (Movie S1), prompting the birds to adjust the timing of their responses. Challenging birds with more complex call patterns allowed us to further test for a capacity to dynamically adjust call timing and predictively avoid jamming by calling in patterns that conform to the presented rhythm. Finally, we tested whether the forebrain song system, which is thought to be dedicated to vocal learning, is required for the control and for the predictive adjustment of call timing. We compared the effects of blocking or inactivating the song system on vocal timing plasticity

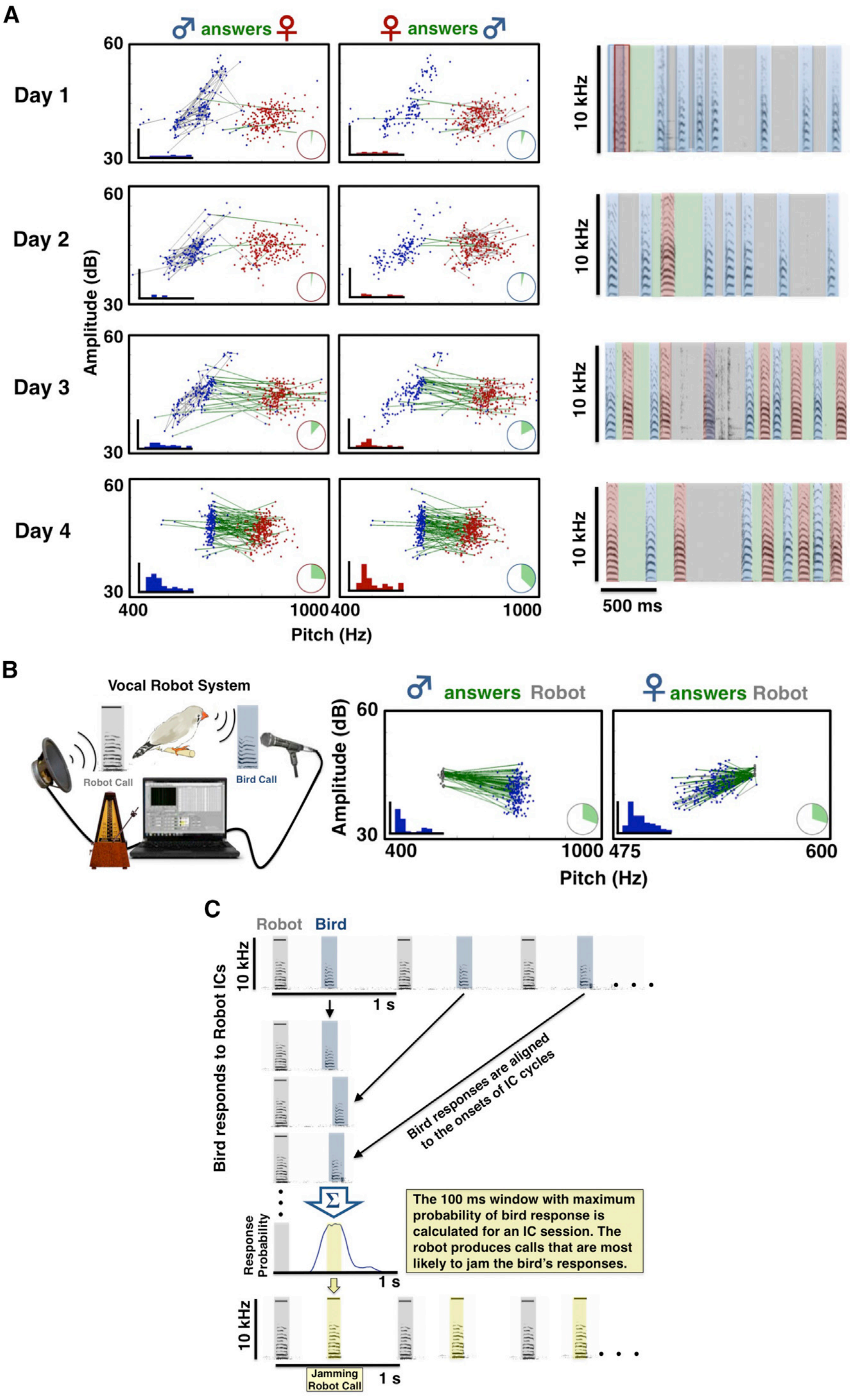

Figure 1. Call Exchanges in a Live Pair and Responses to the Vocal Robot

(A) Calls exchanged between a male (blue) and a female (red) zebra finch over the first 4 days housed together. Dots represent the pitch versus amplitude of short calls. Left: green lines connect female calls to male answers (when answered within 500 ms). Middle: green lines connect male calls to female answers. The insets show histograms of response latencies to partner calls within 1,000 ms. The proportion of partner calls answered is shown. Right: representative sonograms by day. Intervals between calls and answers are shaded in green.

(B) Left: schematic of the vocal robot system. Middle: a male answering robot's 1Hz isochronous calls (ICs). Right: a female answering the robot's ICs (C) The distribution of a bird's call responses to the robot's ICs during a 10 min session are used to compute an optimal jamming window. The robot then produces jamming calls during this window in the following session.

See also Figure S1.

between males and females. Results prompt a reevaluation of the context in which forebrain mechanisms enable vocal plasticity.

RESULTS

Call Interactions

While tracking the development of call interactions between a newly formed pair of birds, we observed that, initially, only a small proportion of calls were answered. Within a few days, however, tightly synchronized vocal coordination [14] emerged, characterized by precise antiphonal calling and higher acoustic stereotypy (Figure 1A). To examine how birds learn to synchronize their calls, we developed a "vocal robot" system (J. Benichov and O. Tchernichovski, 2012, Soc. Neurosci., abstract) that can exchange calls with a bird [19] in modes that are either predictable or adaptive (Figures 1B–1C and Movie S1). Exposing birds to a vocal robot that produces isochronous calls (ICs) at a rate of 1 Hz induced prompt engagement, with birds answering 34% ± 3.7% of ICs during a 10 min session (means ± SEM hereafter; n = 7 males and n = 7 females; Figures 1B and S1). These rates are comparable to those of established pairs within social groups [16]. Response latencies varied across birds (medians ranging between 249 and 466 ms) but were fairly stereotyped for each bird, with 50% of answers occurring within an interval of 249 ± 23 ms. We observed no gender effect on answer latency or stereotypy (n = 7 males and n = 7 females).

Jamming Avoidance

The stereotypy of response latencies provided an opportunity to disruptively mask or "jam" call interactions and determine whether birds can adapt their call timing. After obtaining a reliable estimate of a bird's response latency distribution, we programed the robot to produce call patterns in which each

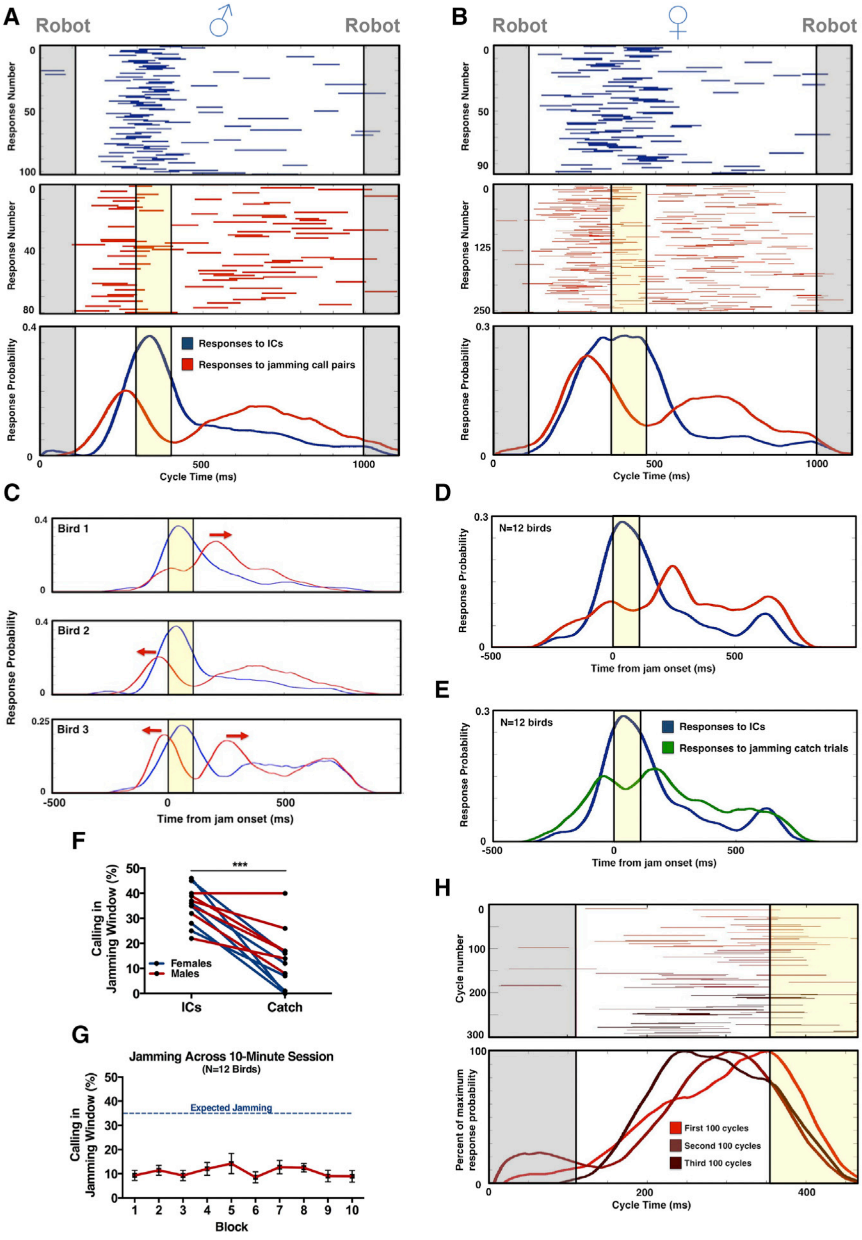

Figure 2. Jamming Avoidance

(A) A male's call responses (blue) to robot ICs (gray). Top: responses are aligned by IC cycle onset and are presented in sequential rows over a 10 min session. Middle: the male's responses (red) to robot call cycles (gray) in which the robot produces jamming calls (yellow) are shown. Bottom: distribution of the bird's responses during the IC session (blue) and during the jamming call session (red) are shown.

(B) As in (A), but for a female.

(C) Examples of jamming avoidance strategies in three birds. Birds' call timing is plotted relative to a normalized jamming window (yellow).

(D) As in (C), but pooled across 12 birds.

(E) As in (D), but comparing call responses across 12 birds for ICs (blue) and catch trials during the jamming session (green).

(F) Birds predictively reduce the proportion of calling within the jamming window during catch trials compared to ICs (n = 12, paired t test, ***$p < 0.001$; percent decrease in six males [red] versus six female [blue], t test, *$p < 0.05$).

(G) Across 12 birds, the average proportion of jamming was lower than expected by chance over the course of a session.

(H) A female gradually changes response latencies over the course of a session (right red, first third; dark red, last third) to predictively call between the robot's jamming call pairs (gray and yellow).

See also Figure S1.

repeating call cycle contains a second (jamming) call exactly when the bird is most likely to respond to the first call (Figures 1C, 2, and S1C). Within a single 10 min session, birds changed the latencies of their responses to avoid jamming (Figures 2A and 2B): they produced calls with shorter latencies, longer latencies, or a combination of shorter and longer latencies (Figures 2C–2E and 2H).

To confirm that this effect is predictive rather than reactive, we incorporated catch trials [20], in which the vocal robot intermittently produced only one call within a cycle (with 10% probability) during a session containing jamming calls (Figure S1C). We could then estimate predictive jamming avoidance by measuring the proportion of responses that fall within the expected jamming window during catch trials (Figure 2E). In 12 birds, we found that the mean proportion of responses within the expected jamming window decreased from 35% ± 2% for ICs to 13% ± 3% during catch trials (Figure 2F; paired t test, $p < 0.001$). This avoidance was statistically significant in males (34% ± 3% to 20% ± 5%; n = 6; paired t test, $p = 0.014$) but greater in females (36% ± 3.5% to 6% ± 2.8%; n = 6; paired t test, $p < 0.001$; t test females versus males, $p = 0.014$), indicating more adaptive timing plasticity in the calling behavior of females. Investigating the time course of changes in answer latencies, we found that birds were able to avoid jamming even during the first 30 s block in a session (Figure 2G), suggesting that, like humans, they can predict the timing of upcoming beats even after hearing only a small number of repetitions [21]. However, we also observed a slower refinement of response timing across a session as some birds learned to fit an increasing proportion of their calls precisely in between jamming call pairs (Figure 2H).

Dynamic Adjustments to Complex Rhythms

Social acoustic environments often contain complex temporal contingencies [22]. We wondered whether songbirds are capable of taking higher order information into account while patterning their communication calls from moment to moment. To determine whether birds adjust their response timing in a generalized way or can alter it dynamically in a context-dependent fashion, we programmed the vocal robot to produce

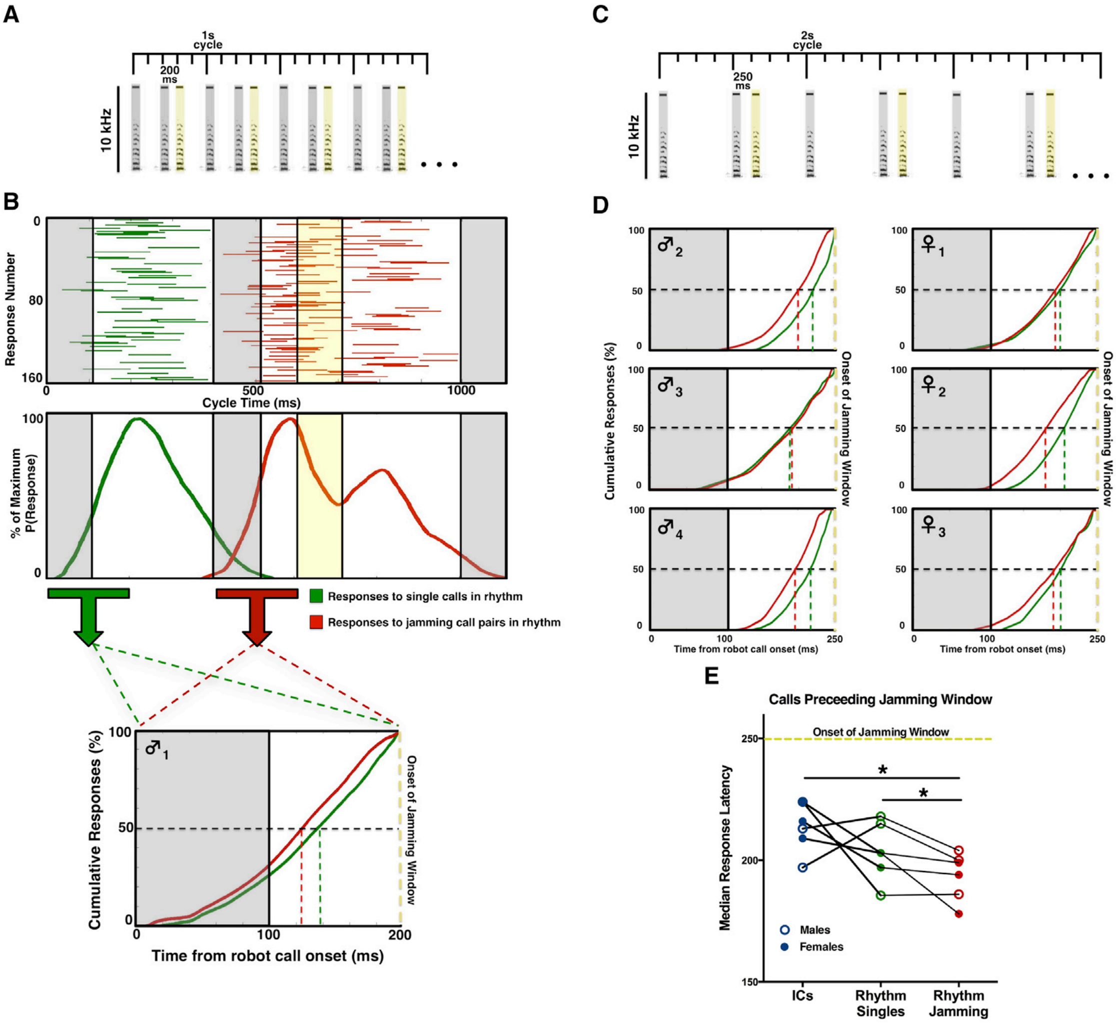

Figure 3. Rhythm Adaptation

(A) A rhythm pattern of rapidly alternating single calls and jamming call pairs (jamming calls in yellow), produced by a vocal robot (1 s cycles, 200 ms jamming latency; see also Figure S2).

(B) Top: a male's call responses to the rhythm pattern shown in (A). Green indicates responses to the single robot calls; red indicates responses to the jamming call pairs. Middle: distribution of bird's responses. Bottom: cumulative responses within the 200 ms following robot call onsets for single calls (green) and the first calls in jamming pairs (red), showing shorter answer latencies prior to jamming.

(C) A robot rhythm pattern as in (A), but with a slower 2 s cycle and 250 ms jamming latency.

(D) Cumulative responses to the slowly alternating rhythm in six birds (green, single calls; red, jamming pairs).

(E) Median response latencies for each bird after ICs (blue), single calls in a rhythm (green), and the calls that precede jamming calls in a rhythm (red; n = 6; paired t tests, *p < 0.05).

jamming calls embedded in complex rhythm patterns. Presenting birds with non-isochronous call patterns, containing alternating jamming and non-jamming elements, we found that birds specifically decrease their answer latencies prior to jamming calls (Figure 3). We observed these dynamic adaptations of latencies within a single 10 min session and over a range of tempos, with cycles of 1–2 s (each cycle is a repeating unit of a single call followed by a pair containing a jamming call; Figures 3A, 3C, and S2A). For example, keeping the one second cycle used before, we programmed the robot to produce a dense pattern of calls (Figure 3A) with alternating call intervals of 400 ms and 200 ms. This way, the bird was presented with longer or shorter alternating windows in which it could answer while avoiding jamming. We could then test whether the bird anticipates the alternating patterns within the 200 ms after the robot calls, immediately before the jamming window. In that 200 ms interval, the bird's median response latency for single calls in the rhythm (calls followed by longer intervals) was similar to that for ICs (singles in rhythm, 140 ± 1.1 ms; ICs, 147 ± 10.6ms). For those rhythmic calls that were followed by a jamming call after 200 ms, however, response latencies were shorter (125 ± 0.8 ms; Figure 3B). Therefore, the bird appeared to alternate between faster and slower responses, conforming to the rhythm produced by the vocal robot.

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To rule out the possibility that this difference between latencies is due to a refractory effect from previous responses to jamming calls, we tested six birds for dynamic adaptation in cycles that were twice as long (Figures 3C–3D and S2B): after single calls in the rhythm, median latencies were similar to those for ICs (single calls in rhythm, 203.6 ± 4.9; ICs, 213.8 ± 4.2; n = 6; paired t test, not significant [NS], p = 0.27). For rhythmic calls that were followed by a jamming call, however, birds accelerated their responses during the same interval, in anticipation of jamming calls (Figure 3E; 193.5 ± 4.0, ICs versus jamming calls in rhythm; n = 6; paired t test, p = 0.022; singles versus jamming, p = 0.026). This predictive response acceleration occurred in both males and females (Figures 3D and 3E), indicating that both can adapt their vocal response latencies in real-time to coordinate with complex rhythms. In contrast to reflexive jamming avoidance responses, such as those seen in electric fish [23], zebra finches used varied strategies to adapt their call latencies in an anticipatory way. This surprisingly high degree of sensory-motor flexibility may suggest pallial (i.e., cortical) involvement.

The Forebrain Song System Mediates Predictive Call Timing

The forebrain song system exhibits premotor activity when males sing, but also prior to the production of unlearned calls [16]. We therefore suspected that the song system is involved in learned timing adaptation. This could be the case in females, too: zebra finch females do not sing, and their song system is assumed to be vestigial [24]. However, because their jamming avoidance and rhythm adaptation performances were similar to—and often better than—those of males, their less anatomically developed song system could perhaps function to guide the coordination of their vocal behavior. In two males and three females, we performed bilateral electrolytic lesioning of the robust nucleus of the arcopallium (RA), which is the common final output of the song system [25]. Lesioning of the RA profoundly affected the timing of responses in all five birds. Although the birds remained responsive to the robot calls (Figures S3A and S3B), the latency of their responses became much less stereotyped (Figures 4A, 4B, and S3C).

To quantify this effect on response time, we calculated a precision measure [26] that estimates deviation from a uniform distribution of response latencies as a *Z* score. After lesioning, the precision of responses to ICs decreases from 4.43 ± 0.37 to 1.91 ± 0.25 (n = 5 birds; paired t test, p < 0.01; Figure 4C, top). Response latency skewness also decreased, from 1.31 ± 0.17 to 0.34 ± 0.13 (n = 5; paired t test, p < 0.01; Figure 4C, bottom), indicating higher symmetry and uniformity of response distributions within a cycle (Figure S3C). Both effects were observed in all three females (Figure 4C). In sum, RA lesions appeared to impair the ability to couple the timing of calls to those of the robot, independent of the overall ability to respond. Birds that underwent identical surgical procedures but received electrolytic lesions outside the song system, at the pallial surface (Figures 4A and 4B; n = 2 females and n = 2 males), showed no changes in timing of their responses (Figures 4C and S4).

The lesion-induced loss of response time precision made it more difficult to target responses for jamming. However, we were still able to test whether the birds exhibited any adaptation to jamming calls by comparing IC responses to those from jamming catch trials. The response probability distributions for ICs versus jamming catch trials, across five lesioned birds, show no decrease in the proportion of calling within the expected jamming window (Figure 4E versus Figure 4D). All five birds showed jamming avoidance prior to, but not after, lesioning (decrease from ICs to catch before, 22.6% ± 3.8%; versus after RA lesions, −1.2% ± 0.6%; n = 5; paired t test, p < 0.01; Figures 4F and 4G).

It is likely that the lesioning of song nucleus RA impacted nearby auditory regions within the archopallium, including nucleus AD (dorsal archopallium) or RA cup. To address this complication, we examined whether the upstream song nucleus HVC, which plays a critical role in song production [27–29], also participates in the regulation of call timing. We recorded from HVC neurons that project to nucleus RA (HVC_{RA}) [30] in three zebra finch males. In all birds, we found neurons that fired while the birds produced contact calls in the presence of a female (Figure S4E). Some of those neurons showed short-latency premotor activity of 10–60 ms prior to the calls. Other neurons, however, showed near-zero latency. To determine whether HVC projections to RA are necessary for regulating call timing, we then disconnected the input from HVC to RA using a procedure that is less likely to compromise auditory regions: we performed bilateral transections of HVC_{RA}-projecting axons [29] in six adult males. In order to verify that projections were fully severed, we injected a retrograde tracer into nucleus RA immediately after the transection. If transections are complete, the tracer should appear only in the anterior forebrain input to nucleus RA (nucleus LMAN [lateral magnocellular nucleus of the anterior nidopallium]), but not nucleus HVC. In three birds, transections were complete, whereas in the other three birds medial projections remained intact, as evidenced by histology and the presence of the tracer in nucleus HVC (Figures 5A and 5B). Interestingly, the three birds that received partial transections showed no impairment in call response precision and were still capable of making adaptive adjustments in call timing to avoid jamming (Figures 5A, 5C, and S4). However, the three birds that received complete HVC_{RA} transections exhibited disrupted call timing, similar to birds with RA lesions, both in the loss of response time precision and reduction of jamming avoidance (Figures 5B–5G). Taken together, our results indicate that although the song system is not required for producing call responses, descending forebrain pathways that include the song system play an essential role in regulating the precise timing needed to predictively coordinate innate calls in both female and male zebra finches.

DISCUSSION

Descending Forebrain Pathways Regulate Anticipatory Call Timing

Unlearned calls are produced by primitive brainstem nuclei in many species. However, our findings show that in zebra finches, regulation of the timing of those calls involves descending forebrain pathways that include the song system. It is unlikely to be a coincidence that the song system is surrounded by auditory centers. Although our investigation focused on the song system, demonstrating activity during calls and loss of function when blocked, anticipatory call timing plasticity is probably driven by the interplay between the forebrain song nuclei and auditory

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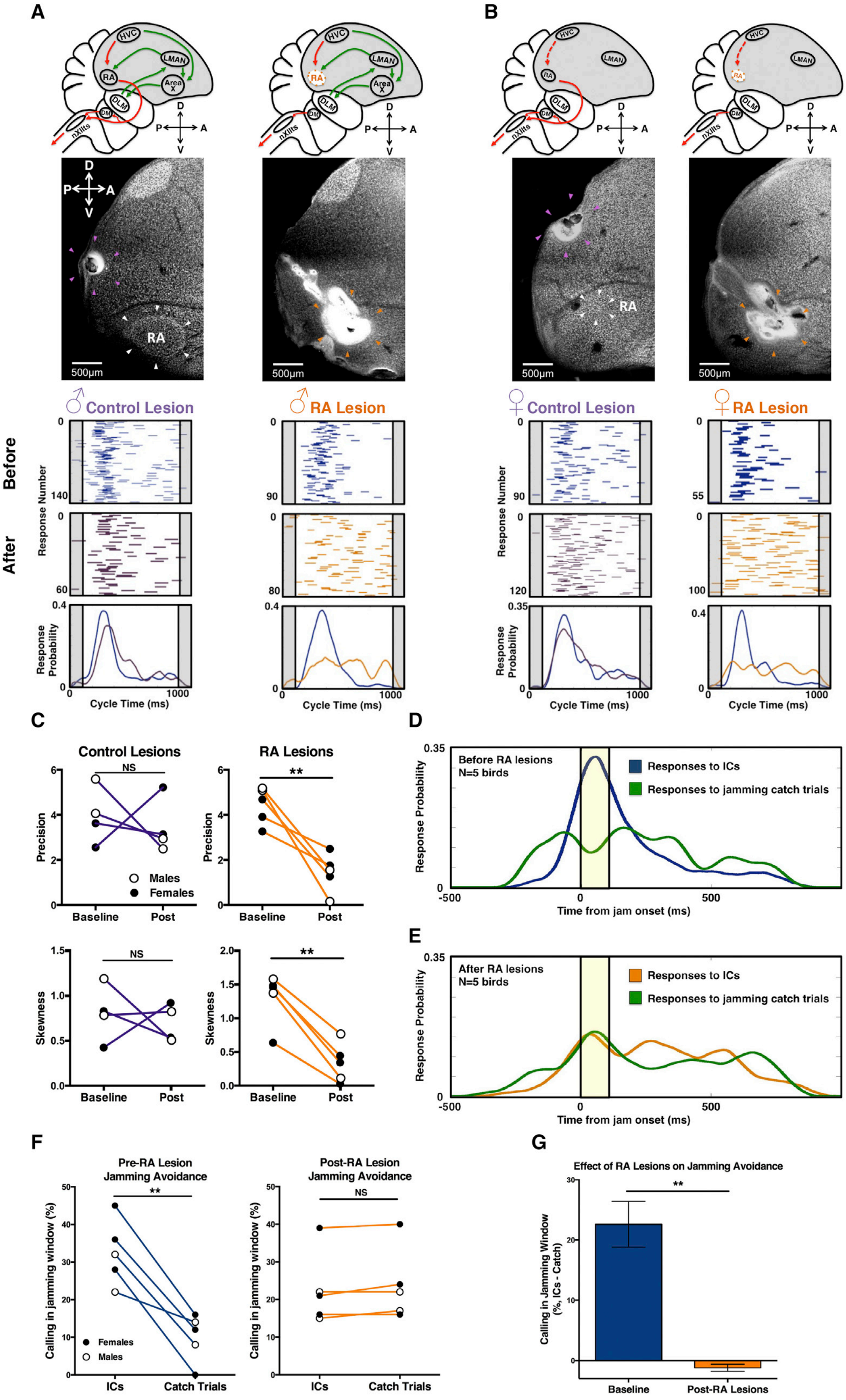

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centers that surround them. Several lines of evidence support this view. First, innate short calls are associated with motor activity in both song nucleus RA [16] and in HVC_{RA}-projecting cells. It would be interesting to investigate whether this activity drives plasticity in vocal timing, perhaps by modulating midbrain and brain stem nuclei, which can generate calls independently of the forebrain [10, 12]. Second, electrolytic lesions of RA and elimination of direct projections to RA from HVC profoundly reduce the timing precision of call responses without reducing response rates. Third, call timing plasticity for jamming avoidance is abolished by either RA lesions or by transection of HVC_{RA} projections. Together, these findings suggest that the precise timing required for coordinated calling is driven by the forebrain song system. However, because birds can anticipate the timing of upcoming jamming calls, even after hearing only a small number of repetitions, the regulation of call timing is likely to also rely on the secondary auditory centers that surround the song system. Interestingly, the descending forebrain auditory pathway, which converges on RA cup, is closely associated with vocal learning [31, 32]. Therefore, RA cup may be involved in the evaluation of timing error or in predicting the timing of the robot's calls. In sum, we suspect that blocking the song system output eliminated the most direct path between the forebrain vocal centers and the primary brainstem vocal motor nuclei (tracheosyringeal portion of the twelfth motor nucleus [nXIIts], nucleus retroambigualis [RAm], and nucleus paraambigualis [PAm]), hence leaving only non-specific tracts that may lack the precision of the direct drive from the forebrain through RA. In this case, midbrain motor centers (most likely the dorsal medial nucleus, DM) and ascending auditory pathways (dorsal lateral nucleus of the mesencephalon, MLd) can apparently still trigger the production of call responses albeit without the predictive timing control provided by the forebrain [11, 33].

The song system's involvement in adaptive call timing suggests that vocal learning and call coordination rely on some of the same mechanisms. In both song learning and call coordination, vocal production changes in reference to a sensory ''template.'' In coordinated calling, the bird does not reproduce the template, but as with syncopation in music [33], timing is shifted in relation to a predicted pattern. Interestingly, the differences between learning to imitate vocal sounds and coordinating calls can explain specific anatomical differences [24, 34–37] between the male and female zebra finch song system: we propose that the female song system functions in call coordination, namely in predictive timing. The male song system functions in vocal learning, which includes both timing and spectral plasticity. Accordingly, song nuclei that are involved exclusively in learning spectral structure should not exist in the female song system. Indeed, the largest song system nucleus, Area X—which is absent from the female brain—is not required for learning time structure in males [24, 38]. In contrast, nucleus HVC, nucleus RA, and RA cup are present in both males and females [10, 12, 24, 37] and have all been specifically associated with temporal aspects of song learning [28, 32, 39, 40]. The song system of the female zebra finch, therefore, appears to be specialized for vocal plasticity with respect to timing.

Phylogenetic Perspective on Vocal Learning

The finding that the female song system can support vocal timing plasticity for coordination has several implications for our understanding of the origins of vocal learning. Although females may only have weak HVC to RA projections (which, as we show in males, can be sufficient for control of call timing), they also have descending connections from HVC shelf to RA cup [39, 41]. This descending auditory pathway is common to vocal non-learners that lack RA (e.g., pigeons) and vocal learners with RA homologs (e.g., budgerigars). However, the descending auditory pathway does not directly project to subcortical vocal production centers in any of these cases. In at least one suboscine vocal non-learning species, the eastern phoebe, this pathway exhibits RA-like premotor properties and does project to brainstem vocal production centers. Lesions to this intermediate structure produce subtle but reliable changes to the unlearned song [7]. From this comparative perspective, it appears that the development of a rudimentary forebrain premotor vocal pathway, in close apposition to the descending auditory pathway, was a pivotal step in the evolution of vocal learning [7].

Evidence that the rudimentary female RA is necessary for the coordination of call timing is consistent with findings by Ter Maat et al. suggesting that the primitive function of nucleus RA might have been to achieve cortical modulation of unlearned vocalizations before it evolved a secondary role in song learning and production [16]. Furthermore, comparative evidence that female singing is ancestral in songbirds [42], suggests that sexual dimorphism in the song system is a more recently derived state. Perhaps, the song system originally functioned in coordinating vocalizations in the context of social bonding [13, 18]. Such a putative ancestral function could also explain why females appear to make greater adaptive adjustments to call timing than males: we propose that the female song system is specialized for regulating vocal coordination. Whereas males retained some of this primitive function, the evolution of territorial singing, might

Figure 4. Effects of RA Lesions on Precision and Jamming Avoidance

(A) Left: control lesion in a male (purple). A control male's responses to robot's ICs (gray) prior to (blue) and after bilateral control lesions (purple) are shown. Right: RA lesion in a male (orange). Experimental male responses to vocal robot ICs (gray) prior to (blue) and after (orange) bilateral RA lesions are shown. Bottom: Overlay of response distributions.

(B) As in (A), but for a control female and an experimental female.

(C) Precision and skewness of responses to ICs in two males and two females before and after control lesions (n = 4; paired t tests, NS, $p > 0.65$) and in two males and three females after bilateral RA lesions (n = 5; paired t tests, $^{**}p < 0.01$).

(D and E) Response distributions for ICs and catch trials pooled over five birds (as in Figure 2E) before (D) and after (E) RA lesions.

(F) Percentage of calling within the jamming window for ICs versus catch trials before (blue; n = 5; $^{*}p < 0.01$) and after RA lesions (orange; NS).

(G) RA lesions abolish jamming avoidance, measured as the difference in percent of calling within jamming window for ICs and catch trails (n = 5; means ± SEM; $^{**}p < 0.01$).

See also Figures S3 and S4.

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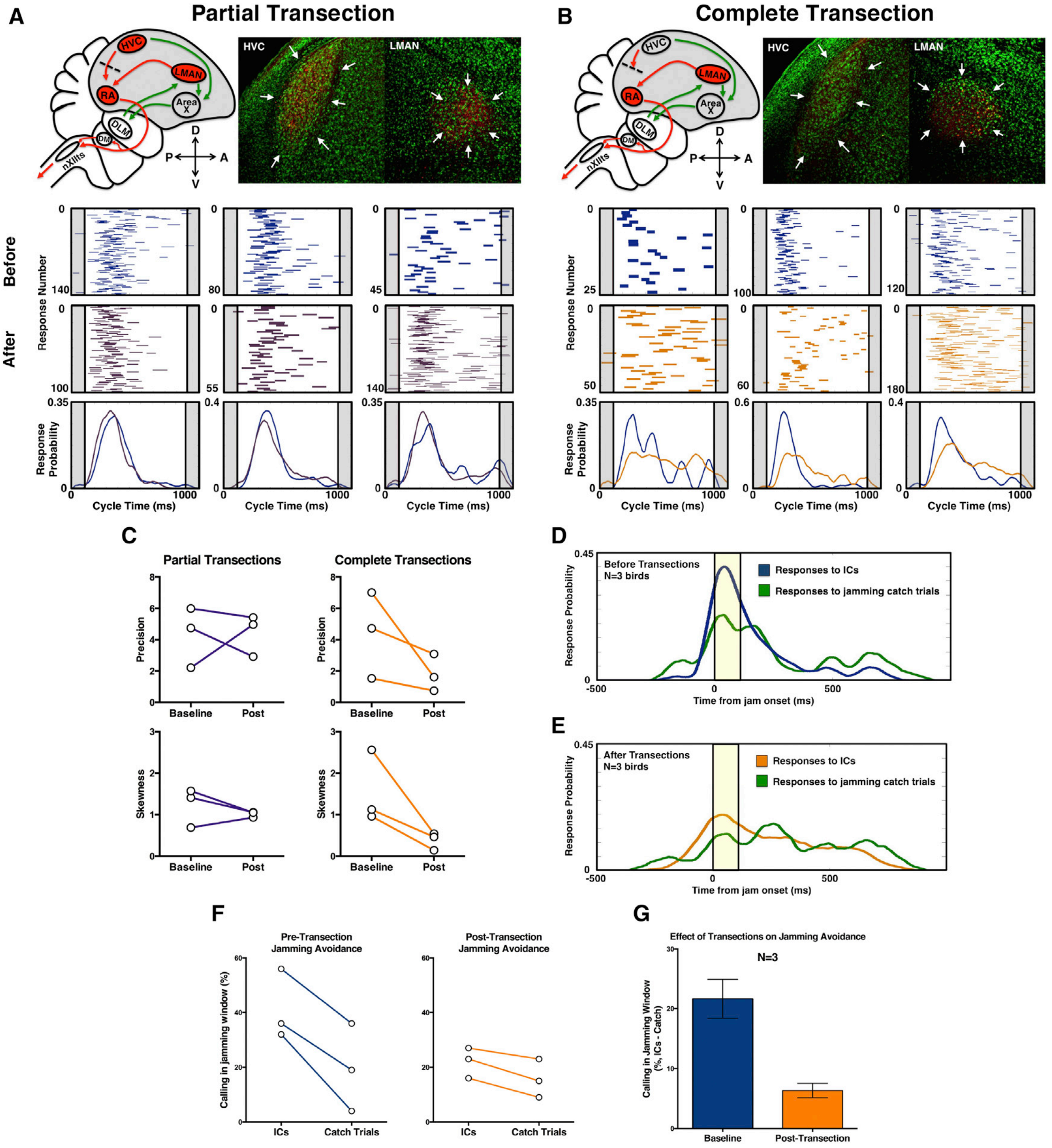

Figure 5. HVC Activity Associated with Call Production and the Effects of HVC_{RA}-Projecting Axon Transections

(A) Partial transections of HVC_{RA} fiber tracts (medial HVC_{RA} tracts intact) allowed retrograde tracer (injected in RA) to reach RA-projecting cells HVC and LMAN. Responses to ICs before (blue) and after (purple) partial transections (n = 3) are shown.

(B) Complete HVC_{RA} transection, verified by absence of tracer within HVC and presence of tracer in LMAN. Responses to ICs before (blue) and after (orange) complete bilateral transections (n = 3) are shown.

(C) Complete transections results in decreased precision and skewness of responses to ICs (n = 3 birds). Precision is unaffected in transected birds with intact medial projections (n = 3).

(D and E) Response distributions for ICs and catch trials (as in Figure 4F) pooled over three birds before (D) and after (E) complete transections.

(F and G) As in Figures 4F and 4G, respectively. The percent of calling within jamming window for ICs versus catch trials before and after complete transections of HVC_{RA} projections (F) and (G) reduction of jamming avoidance after complete transections (G) are shown.

conflict with coordination. Perhaps the tendency of males to "stand their ground" explains their inferior performance in the jamming avoidance tests.

Vocal learning and auditory-motor synchronization (e.g., dancing to music) are considered to be evolutionarily and mechanistically linked, perhaps stemming from shared social origins [43–45]. Our results provide direct evidence for a similar link between song learning and call synchronization, except that here both phenomena are vocal and are supported by the same sensorimotor pathway. In human beat perception, premotor cortex is implicated in the analysis and prediction of sound timing regularities [33, 43]. Evidence for an analogous process in the song system is emerging from recordings from interacting zebra finches (S. Ma et al., 2015, Soc. Neurosci., abstract), as well as the coordinated duets of male and female plain-tailed wrens, in which pre-motor activity may encode shared information about a rapidly alternating partner's timing intervals [46]. The loss of timing precision and jamming avoidance after blocking the song system in zebra finches may be the consequence of disrupting such an auditory-motor timing prediction mechanism that guides call coordination.

The remarkable vocal coordination capacity that we observed in both female and male zebra finches, using controlled behavioral assays, supports the idea of a mechanistic continuum from vocal coordination to vocal learning, spanning a functional gap that has separated vocal non-learners from vocal learners. Considering that coordination of unlearned vocalizations, without forebrain control, is relatively widespread across taxa, the likely primitive function of the forebrain song system was to enable plasticity in the timing of social behavior. Such a general-purpose sensorimotor communication system could facilitate vocal interactions on different timescales: in the case of calls, it enables predictive modulation of vocal timing, whereas in the case of learned songs, plasticity extends to developmental changes in vocal structure. In this scenario, the ability to make predictive behavioral adjustments from moment to moment, with respect to a social partner, provided the mechanistic basis for vocal learning (i.e., "offline" coordination with the auditory memory of the vocalizations of others). This social coordination hypothesis can also begin to explain some of the evolutionary convergence in the mechanisms of vocal learning and auditory-motor synchronization seen across mammalian and avian brains [6–8, 33, 43–45].

EXPERIMENTAL PROCEDURES

Animal Care

All experiments were performed in accordance with guidelines of The US NIH and have been reviewed and approved by the Institutional Animal Care and Use Committees of Hunter College of the City University of New York and New York University Langone Medical Center.

Analysis of a Synchronized Pair

Call interactions were recorded continuously using Sound Analysis Pro 2011 (SAP). Birds' call onsets and durations were identified semi-automatically and were analyzed using MATLAB 7. Call feature calculation and cluster analyses were performed using SAP. Cluster information was used to elucidate bird identity. We then used MATLAB 7 for analysis of call answers (calls within a 500 ms window) for 500 consecutive calls, selected pseudorandomly from each of the 4 days.

Vocal Robot

We developed an interactive vocal robot application in LabVIEW (Supplemental Experimental Procedures; code is available upon request), which delivers patterns of calls at programmable intervals that can be tailored (manually or automatically) to match (or avoid) answer latencies of individual birds (Figures 1, S1, and S2 and Movie S1).

Computation of the Jamming Window

Each bird's calls were recorded over a 10 min session of isochronous robot calls and were used to calculate the jamming window. Call response onsets and offsets were coded relative to the onset of the previous robot call. These onsets and durations were summed across all cycles in a session to produce a response probability distribution. The jamming window was defined as the 100 ms interval with the highest response density. The window onset is the latency of the jamming call delivered in each 1 s cycle during the next session of jamming calls. Jamming percentages were calculated as the proportion of total calling activity falling with the bounds of the jamming window. Catch trials were calculated as above. Response latencies and skewness were calculated in MATLAB 7 using the onsets of responses relative to the previous robot call. For responses to single calls and jamming calls in a rhythm, we calculated median latencies for responses with onsets prior to the expected jamming window.

Precision Score

Precision scores were calculated as in [26] for each session using the proportion of all response onset latency differences that were within ±50 ms (approximate duration of a call). This proportion was used to compute a Z score relative to a distribution of proportions from 1,000 simulated sessions containing an equal number of uniformly distributed pseudorandom latencies. The precision score is expressed as the square root of this Z score.

RA Lesions

Electrolytic RA and control lesions were performed bilaterally in anesthetized males and in females.

Electrophysiological Recording in HVC

Intracellular recordings from four antidromically identified $\mathrm{HVC_{RA}}$ neurons in three freely behaving adult males were obtained using a motorized intracellular microdrive. A detailed description of this approach can be found in [30].

Transection of HVC to RA Projections

Transection of axonal projections from nucleus HVC to nucleus RA were performed bilaterally in adult males (as described in [29]).

Also see the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.12.037.

AUTHOR CONTRIBUTIONS

J.I.B., O.T., M.A.L., S.E.B., and E.G. designed the research. J.I.B., S.E.B., and D.V. performed experiments. J.I.B., S.E.B., D.V., E.G., M.A.L., and O.T. analyzed data. J.I.B. and O.T. wrote the original manuscript, and all authors helped revise it.

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